avenues of AFM study - has previously only been achieved in highly isolated non-biological environments (i.e., cryogenic temperatures, ultra-high vacuum). We adapted techniques originally developed by the optical trapping microscopy community, and have constructed an ultra-stable AFM in which the tip and the sample positions are independently measured by, and stabilized with respect to, a pair of laser foci in three dimensions. Recently, we have extended ultra-stable AFM to common biological imaging conditions (tapping mode in aqueous buffer solution) and have exploited local observation of the three dimensional (3D) tip trajectories to yield 3D interaction force components in a direct manner. In this talk I will discuss these developments in the context of addressing central questions in membrane biophysics.

836-Pos Board B616

Fast and Accurate Photodiode-Based Detection of Multiple Trap Optical Tweezers with Crosstalk-Elimination

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Multiple trap optical tweezers have proven instrumental for a variety of exciting biophysical discoveries, e.g., from shedding light on molecular motor mechanisms to revealing intricate details of DNA-protein interactions. This 'second' generation of optical tweezers allows for advanced trapping geometries beyond single traps, however, these increased manipulation capabilities typically come at the price of more challenging position and force detection. The accuracy of position and force measurements is often compromised by crosstalk between the detected signals, this crosstalk leading to systematic and significant errors on the measured forces and distances. We developed an easy-to-implement simple method that enables simultaneous three-dimensional tracking of several individual objects in dual trap optical tweezers [1] and multi-trap holographic optical tweezers [2,3]. The method is based on spatial filtering and is highly compatible with standard back-focal-plane photodiode-based detection offering unrivaled temporal and spatial resolution with minimal crosstalk. This fast and accurate photodiode-based detection offers distinct advantages over camera-based solutions and opens the possibility for a variety of new biophysical assays. The reported technique significantly improves the accuracy of force-distance measurements, e.g., of single molecules, and hence provides much more scientific value for the experimental efforts.

Publications:

[1] D. Ott ,S.N.S. Reihani, & L.B. Oddershede, ''Crosstalk elimination in the detection of dual-beam optical tweezers by spatial filtering,'' Review of Scientific Instruments,(2014).
[2] D. Ott ,S.N.S. Reihani, & L.B. Oddershede, ''Simultaneous three-dimensional tracking of individual signals from multi-trap optical tweezers using fast and accurate photodiode detection,'' Optics Express (2014).

Filed Patent:

[3] L.B. Oddershede, D. Ott , S.N.S. Reihani, ''A detection system for an optical manipulation system for manipulating micro-particles or nano-particles of a sample by means of at least two optical traps,'' PA 2014 70097, Denmark.

837-Pos Board B617

Precise Partition of Micro/Nanoparticles in an Electro-Optofluidic Platform

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Integrated optofluidics, when controlled electro-optically, can facilitate the high-throughput on-chip manipulation and detection of biomolecules and micro/nanoparticles. Previously we demonstrated stable trapping and precise manipulation of trapped microspheres, with positions of trapped microspheres dictated by antinodes of a standing wave formed along a nanofabricated waveguide [1]. Using this nanophotonic platform, we now report a method of generating an optical binding effect on-chip. We show that an array of microspheres can be stably trapped and uniformly spaced with a travelling wave. The direction of the travelling wave is rapidly switched using the thermo-optic effect via an integrated electric microheater which modulates the phase of a wave in a waveguide in a Mach-Zehnder interferometer. Our theoretical work suggests that the periodic trapping potential is a result of multiple-interference of the wave due to its interactions with the microspheres. Such a demonstration of on-chip optical binding provides a novel mode of manipulation and spacing of trapped micro/nanoparticles on an optofluidic platform.

[1] M. Soltani, J. Lin, R. A. Forties, J. T. Inman, S. N. Saraf, R. M. Fulbright, M. Lipson & M. D. Wang, Nanophotonic trapping for precise manipulation of biomolecular arrays. *Nature Nanotechnology* **9**, 448-452 (2014).

838-Pos Board B618

Transverse Magnetic Tweezers for Direct DNA Extension Measurements

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Magnetic tweezers are an important tool used to stretch and twist single DNA molecules. While longitudinal magnetic tweezers have been widely used for DNA extension and rotation measurements, transverse MTs have found more limited applications due to the lack of a method to directly measuring DNA extension, even though they are better suited for visualization of events taking place on DNA. Here, we present a transverse magnetic tweezers setup that can directly measure DNA extension. This method should provide a more accurate determination of the anchoring position of the DNA on the substrate. It simplifies the measurement of the DNA extension and is potentially useful for studying short DNA constructs. In addition, our external lateral magnet can rotate, allowing us to twist torsionally constrained DNA at a constant force to generate DNA supercoils.

839-Pos Board B619

Human Red Blood Cell Adhesion to Laminin Measured by Atomic Force Microscopy

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Introduction: Red blood cells (RBCs) express several surface adhesion receptors which modulate cellular function and mediate cell-cell interactions, or adhesion, on both healthy and pathological RBCs. In this study, we established an in vitro single-cell force spectroscopy (SCFS) technique to study human RBC adhesion to substrates coated with laminin protein. Additionally, in order to establish the sensitivity of the assay, we investigated changes in the detachment force between single RBCs and the laminin substrate in the presence of pharmacological modulators of the cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA) pathway.

Methods and Results: Using blood obtained from healthy human subjects, we recorded adhesion forces from single RBCs attached to AFM cantilevers as the cell was pulled-off of substrates coated with laminin. SCFS records force-distance curves of an approach/retraction cycle between the RBC probe to and from the laminin-functionalized substrate. We found that an increase in the overall cell adhesion measured via SCFS is correlated with an increase in the resultant total force measured on 1 μm2 areas of the RBC membrane via single-molecule force spectroscopy (SMFS). Finally, RBCs were treated with the PKA activator-forskolin (FSK), PKA inhibitor-KT5720 and epinephrine to study the variations in the adhesion of BCAM/Lu to laminin due to pharmacologic modulation of the cAMP signaling pathway.

Conclusion: We established that SCFS can detect variations in the detachment force of RBCs to laminin based primarily on the number of active BCAM/Lu receptors, which was modulated via biochemicals affecting the cAMP-PKA pathway. This study shows important implications for AFM-based SCFS measurements in understanding and evaluating the pharmacologic response of adhesion receptors on RBCs. Further, this method can easily be employed to measure the adhesive interactions of various cell types with functionalized substrates or other cells.

840-Pos Board B620

Atomic Force Microscopy (AFM) Analysis of the Bacterial Polar Protein Popz

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Structural studies of filament-forming biomolecules have classically relied on optical imaging methods such as fluorescence and electron microscopy. While these methods have yielded novel insights into mechanisms of macromolecular assembly, they often require perturbative labeling or staining procedures that can affect the assembly process. In contrast, Atomic Force Microscopy (AFM) has emerged as a powerful imaging tool to directly study native nanostructures at high resolution without labeling. Unlike optical methods, AFM utilizes a nano-scale cantilever to generate a topography map of surface-immobilized molecules in air or in aqueous environments, allowing studies under more physiological or dynamic conditions. However, like all imaging methods, most biological samples require electrostatic or covalent interaction

of the biomolecule with a surface for imaging, and care must be taken to avoid surface-specific effects on the sample. Here, we examined the structural organization of the bacterial polar scaffold protein PopZ by AFM, and analyzed the effect of surface-immobilization conditions on PopZ's nanostructural assembly characteristics. We measured the structures and densities of PopZ complexes on positively and negatively charged as well as hydrophobic surfaces, and compared structural organization of these assemblies in aqueous environments. Our results illustrate how choice of surface immobilization conditions can affect structural studies of polymeric assemblies, and demonstrate the tremendous advantages of AFM for directly imaging biomolecules in aqueous, physiological conditions. Finally, our results provide new insight into the structures of multimeric PopZ nano-assemblies that have been thus far unattainable using standard EM methods, providing direct evidence for PopZ self-assembly into organized three-dimensional polymeric networks.

841-Pos Board B621

Atomic Force Microscopy of Protein Translocation Machinery in Supported Lipid Bilayers

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More than 30% of proteins in any organism are transported from the site of synthesis into or through cell membranes to properly localize and function. The general secretory (Sec system) is the major route of export for proteins from the cytosol of Escherichia coli and all eubacteria. The pathway through the membrane - the translocon - is provided by SecYEG, a protein complex that is highly conserved having homologs across the kingdoms of life. SecA is the ATPase of the Sec system and it binds SecYEG to perform translocation. In so doing, SecA makes large surface area contact with the unstructured cytoplasmic loops spanning transmembrane helices 6-7 and 8-9 of SecY. Despite their broad functional significance, measurements of flexible and disordered protein domains remain a significant experimental challenge. Recently, atomic force microscopy (AFM) has emerged as an important complementary tool in biophysics and is well suited for studying membrane proteins in near-native conditions. Here we studied purified SecYEG that was reconstituted into liposomes. After confirming activity, changes in the structure of SecYEG as a function of time were directly visualized. The dynamics observed were significant in magnitude and were attributed to the aforementioned loops of SecY. In addition, we identified a distribution between monomers and dimers of SecYEG as well as a smaller population of higher order oligomers. We have also imaged SecA engaged on SecYEG and related the structural states observed to the activity of the translocase. Currently we are working towards determining the oligomeric state of SecA during active translocation, and further exploring the dependency which we uncovered of the SecYEG oligomeric state on the protein species being transported. Taken together, this work provides a novel and near-native vista of central components of the protein translocation machinery.

842-Pos Board B622

A Novel AFM-Based Technique for Measuring Coupled Phenomena in Cardiac Myocytes

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In vitro investigation of mechano-electrical behavior of cardiomyocytes can open new ways for detecting and predicting pathological conditions directly linked to alteration of the Excitation Contraction Coupling (ECC), such as heart failure, myocardium ischemia, cardiac hypertrophy and genetic dystrophies. For this purpose we propose an integrated experimental setup based on atomic force microscopy (AFM), extracellular field potential measurement by microelectrode arrays (MEAs), and fluorescence microscopy (FM), that can supply time-resolved information, at the single fibre level, of cell mechanical properties, in relation with its electrical and chemical properties, during the beating cycle. AFM allows to probe topography and visco-elastic properties of beating myocytes with sub-micrometric resolution that can be coupled to simultaneous calcium imaging by FM and electrophysiology recording by MEA. The use of MEA as a multisite, non-invasive, recording technique greatly increases the throughput of a single experiment. In order to explore the capabilities of the proposed experimental method we monitored ECC and its alterations as a response to the introduction of substances altering cell contractility, such blebbistatine, or beating rate such as caffeine.

843-Pos Board B623

Cadherin Conformational Shuttling Captured Using Single Molecule Atomic Force Microscopy

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Classical cadherins are Ca^{2+}-dependent cell adhesion proteins that play essential roles in the development and maintenance of tissues. Cadherins bind in two distinct conformations, Strand Swap dimer (SS-dimer) and X-dimer. These conformations respond to mechanical force differently: while SS-dimers form slip-bonds which become weaker as the force is increased, X-dimers form catch-bonds that strengthen when pulled. In contrast, wild type cadherins form ideal bonds, which are insensitive to applied forces. Here we use single molecule Atomic Force Microscope (AFM) force clamp spectroscopy and computer simulations to resolve the molecular mechanism for ideal bond formation. Using single molecule force clamp measurements, we demonstrate that ideal bonds are formed as wild type cadherins shuttle between X-dimer and SS-dimer conformations. Computer simulations show that the probability of conformational switching varies with force. Our data suggest that force induced conformational shuttling enables cadherins to withstand mechanical stress.

844-Pos Board B624

Whole Cell Biochemical and Nanomechanical Investigations of Bacillus Using Atomic Force Microscopy

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Atomic force microscopy (AFM) based adhesion force spectroscopy and elasticity measurements have emerged as powerful tools in the biophysical analysis of cellular systems. Such measurements can now be extended to probe the distribution of specific biomolecules and elasticity at the single cell level. Here, we report on studies using Bacillus cereus, a common food-borne pathogen, as a model system. Using AFM-based adhesion force spectroscopy coupled with lectin probes - wheat germ agglutinin (WGA) and concanavalin A (ConA), we show the spatial mapping of specific cell-surface carbohydrate targets - N-acetylglucosamine (GlcNAc) and mannose/glucose (Glu). We show the compositional change from the vegetative cell to the spore, mapped, and quantified at the nanoscale across single B. cereus cell surfaces. The surface molar ratios of GlcNAc:Glu are ~4:1 on a vegetative cell surface but display a switch to ~1:3 on a spore surface. This trend is in excellent agreement with previously reported values using GC-MS and chromatography conducted on bulk samples. Further, we investigated the morphological and nanomechanical transformation of B. cereus in the sporulation process in response to temporal nutrient deprivation conditions. Using AFM imaging and elasticity mapping, we observed the morphogenesis and the progression in elasticity of the endospore and released mature endospore. The elastic modulus increased nearly 300% from the rod-like vegetative cell (1.1 ± 0.2GPa) to the oval-shape mature spore (5.1 ± 0.3 GPa) due to the formation of spore coat and cortex. Collectively, these investigations demonstrate atomic force microscopy as a versatile single cell technique in microbiology to quantitatively detect and spatially map bacterial surface biomarkers and probe key spatial and temporal changes in surface biochemical and nanomechanical properties during cellular activities.

845-Pos Board B625

Effect of Surface Density of Active Sites on Rupture Force Distributions of Single Molecule Interactions

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Atomic force microscopy (AFM) is used in measuring dissociation in protein systems and protein-protein interactions forces at single molecular level. However, an explicit interpretation of the acquired rupture force data is not always easy. The Bell-Evans Standard Theory, used for analyzing rupture force data (contingent on the concept of thermal activation and the deformation of the activation barrier) yields a rupture force distribution function which is skewed to the left (towards low force). However, most of the experimental measurements of rupture force data generate a probability distribution function (pdf) with a high force tail. The probable cause of this high force tail in the rupture force pdf is either multiple attachments (though recognizable multiple ruptures are typically removed from rupture force analysis) or heterogeneous bonding. To study the effect of multiple attachments, we created a varying density of active sites using self assembled monolayer by incubating the substrate in mixed solutions of active (biotin) and inactive (methyl-terminated) PEG molecules and pursued imaging and force measurements with avidin functionalized